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(1)

A METHOD FOR THE COLLECTION OF SWEAT AND ITS ANALYSIS IN THE NORMAL ADULT.

A THESIS

PRESENTED IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE
MASTER OF MEDICAL SCIENCE

BY

CHARLES ARTHUR COLTMAN, JR., M. D.
CAPTAIN UNITED STATES AIR FORCE (M.C.)

OHIO STATE UNIVERSITY
1963.

$7.60

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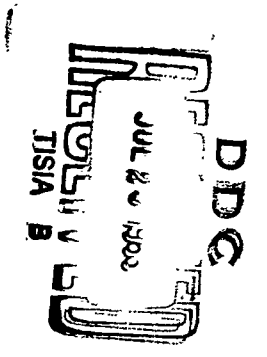

INTRODUCTION

The original discovery[1] of the electrolyte abnormalities of sweat in cystic fibrosis of the pancreas was preceded by several thorough investigations[2,3] of the genetic nature of the disease. There have been many subsequent studies[4,5,6,7,8,9], attempting to understand the frequency with which the gene for cystic fibrosis occurs in the general population as well as to identify the heterozygous expression of the disease. The sweat test has been used as a relatively unstandardized tool in the evaluation of the relationship of cystic fibrosis to adult medicine.

An attempt is [redacted] made to review the information available to date on the genetics of cystic fibrosis relative to adult disease. The use of the sweat test as a diagnostic tool in adults is evaluated and the limitations pointed out. A method for the collection and analysis of sweat electrolytes is described and applied to a study of a large population of normal adults.

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GENETICS:

The familial nature of cystic fibrosis of the pancreas was first described by Andersen & Hodges[2] in 1946 when they pointed out that the incidence in siblings, in a study of 47 families, was approximately 25% as expected by Mendelian-recessive genetics. Because of the distribution of cases in the families, they felt that the disease required more than one factor for its expression. In 1949 Lowe et.al[3] statistically analyzed the data obtained from a study of 143 patients with cystic fibrosis of the pancreas and found 30 cases, always born to normal parents, with an equal distribution of boys and girls. He concluded that the disease was inherited as a single Mendelian-recessive trait. Others[4,10,11,5] are in agreement.

In order to evaluate the frequency of the gene in the population, and thus analyze the incidence of heterozygous carriers, it is necessary to determine the frequency with which the homozygous cystic fibrosis disease occurs. Lowe et.al.[3] made a rough estimate of the case rate, lying somewhere between 1 case per 100 and 1 case per 10,000 births. Goodman & Reed[11] analyzed autopsy data from 19 hospitals and found that 3% of hospital deaths were due to cystic fibrosis of the pancreas. He suggested that the range of frequency of cystic fibrosis is between 0.86 to 1 case per 1,000 births. Childs[5] felt that the incidence of the disease was somewhere between 1 per 1,000 and 1 per 10,000 live births. Steinberg & Brown[4], in a careful analysis of the incidence of cystic fibrosis in the state of Ohio, concluded that the incidence was 1 in 3700 births, but were unable to account for the marked infrequency of the gene occurring in the Negro

race.

Salam & Idriss[6] reported five cases of 10,000 children seen at the American University of Beirut, or 1 in 2,000. Houstek & Vavrova[7] found the incidence of the disease in Czechoslovakia as one case among 2,604 live-infant births. Selander[8] places the incidence considerably lower at 7 per 10,000 births in Sweden. The most recent, and as yet inconclusive evidence, has been derived from the data of Merrith and Hanna,[9] who devised a formula for calculating gene frequency by determining the frequency with which cystic fibrosis is found in first cousins of patients with cystic fibrosis. They observed three affected first cousins in 74 families of a total of 332 first cousins and calculated the incidence as 1 in 1400.

Most of the available information suggests that the best figure lies between 1 in 1,000 and 1 in 3,000 live births, with some racial and geographic variation. If one were to consider, for the sake of discussion, that the frequency of cystic fibrosis in white children in the United States is 1 in 1,000, then the gene must occur in the population in about 1 in 20.[12] With such a high gene frequency in the population, one is prompted to ask two questions. How is a gene, which in the homozygous state produces "genetically lethal" disease, maintained in the population in such high frequency? If it does, in fact, occur in high frequency in the population, how is it manifest in its heterozygous state?

There are at least three possible explanations for maintenance of a "genetically lethal" gene in the population in high concentration under these circumstances. Consanguinity, occurring more frequently

among the parents of children with cystic fibrosis, and thus producing the homozygous state at an increased rate, is one consideration. Lowe et.al.[3] found only one case of consanguinity in 118 families and concluded that since this was a rate indistinguishable from that in the general population, consanguinity was not a factor.

A very high mutation rate could account for the high gene frequency. Goodman & Reed[11] point out that the mutation rate for chromosome per generation is equilavent to the frequency of lethal children in the population. Consequently, the possible mutation rate for normal to abnormal genes for cystic fibrosis of the pancreas is between 0.7×10^{-3} and 1.0×10^{-3}, a very high mutation rate compared with those which are well established with lower organism. Childs et.al[5] calculated the mutation rate at 1 per 1,000 or 1 per 10,000 mutation per locus per generation, a situation outside the experience with many other human disease.[5,10] Most known human mutation rates have frequencies less than 1 per 25,000, making this an unlikely cause of the high gene frequency.

The final alternative is that a selective advantage is conferred upon the heterozygote over the homozygous normal persons.[4,13] Steinberg & Brown[4] calculated that if cystic fibrosis were to occur with a frequency of .001, the gene frequency would approximate .031. Under these circumstances the gene could be maintained in the population with a selective disadvantage to the homozygote normal, relative to the heterozygote, of approximately .03; that is to say, for every 100 offspring left by heterozygotes, 97 are left by heterozygote normal individuals. Such a reproductive difference could be

detected in a large sample with some difficulty. Their studies show that in Ohio the incidence of cystic fibrosis is 0.000267 with a postulated gene frequency of approximately 0.016. "A lethal gene, occurring in this frequency, may be maintained in the population and in equilibrium, in the absence of mutation, with a heterozygote advantage of approximately 1.6%, a difference which could be impractical to measure in a society such as ours."[4] It has been suggested[14] that such "heterozygotic vigor" resulting from improved resistance of the heterozygote to staphlococcal infection exists, accounting for a selective advantage and making it a likely explanation for the frequency of this gene.

It should be pointed out that there are certain disagreements as to the genetics of this disease. Koch et.al.[15] found approximately 84 patients with cystic fibrosis who were adults and observed that large numbers of relatives of 41 of these 84 patients also exhibited clinical features of cystic fibrosis, concluding that the gene was transmitted by an autosomal dominant inheritance with "high penetrance and variable expressivity." Roberts[16], in a study of 73 families found not only that the incidence was 1 in 500 live births, but that there were too many affected children to satisfy the quarter ratio hypothesis of recessive inheritance.

The gene undoubtedly occurs in the population with extreme frequency and the second major question deals with the detection of the heterozygous state in the population. In his initial genetic studies, Lowe et.al[3] pointed out that, while in a cystic fibrosis family neither parent nor sibling is affected, they may carry the

recessive character, but unless stressed will never be suspected of doing so. The abnormal sweat test which has been used as an excellent technique for the diagnosis of the disease in the homozygous state has been somewhat less satisfying in detecting the heterozygote. Childs et.al.[5] suggests that the sweat test performs this function with distressing infrequency in the heterozygote. Smoller & Hsia[17] studied the sweat sodium concentration in families of children with cystic firbrosis and in normal controls. He found that 95% of the normal controls had a sweat sodium which was less than 50 mEq/L. Using this as his upper limit of normal, he found that one-half of the parents and one-third of the unaffected siblings studied had sweat concentrations beyond this limit and were considered to be demonstrable heterozygotes. They[17] were unable to demonstrate such an aberration in the remaining parents and siblings, who undoubtedly represented an expression of the heterozygous state, but the proper stress required to bring out the complete expression had as yet, not been determined. Di Sant'Agnese & Andersen[12] studied the parents and siblings of cases of cystic fibrosis, again using 50 mEq/L as the dividing line between normal and abnormal concentrations. Seventeen per cent of parents and twenty-nine per cent of asymptomatic siblings of patients known to have cystic fibrosis had abnormally high sweat electrolytes. He agreed that the sweat aberration was probably a reflection of the heterozygous state but that some as yet unknown fundamental defect has not yet been elicited. There are a large number of patients carrying the recessive gene, who have concentrations of sweat electrolytes well within normal limits, even when the upper limits of normal are placed so low as

50 mEq/L.

RELATIONSHIP TO ADULT DISEASE:

The interest of the internist becomes manifest when considering three aspects of the problem of cystic fibrosis of the pancreas. The first deals with the fact that, as a result of effective antibiotic therapy, more active interest in the disease, and the development of specialized treatment centers, the life of patients with cystic fibrosis is becoming prolonged and there are an increasing number of adolescents and young adults presenting with the disease. Secondly, there is a large and ever-increasing number of adult patients being reported who have a illness which is clinically indistinguishable from the homozygous disease, which we previously had known as existing only in children.[12,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32] Finally, in quest of the heterozygous carrier, studies in the measurement of the electrolyte composition of sweat in adults have been energetically pursued. Patients, having been found with the association of a well-known adult disease and increased salt content of sweat, are said to have some forme furste of the disease; such as chronic lung disease,[33,34] peptic ulcer,[15] allergy[3,36,37,38,39,40] hepatic cirrhosis,[41,42] and diabetes mellitus[43,44,45,46,47,48,49]. These problems facing the internist are real ones and deserve our consideration.

There appears to be a little question that there is some, as yet undefined, relationship between cystic fibrosis of the pancreas and certain well-known adult disease states. Peterson[33] found fourteen patients out of 262 subjects who had abnormal sweat tests, t en of

whom had severe endo-bronchial disease and four additional patients, two with peptic ulceration, one chronic pancreatitis and one allergic bronchitis. A recent flurry of literature demonstrates a more than casual relationship between cystic fibrosis and diabetes mellitus. Rene & Kelly[44] found a family history of diabetes mellitus in eleven of twenty-five patients with cystic fibrosis and felt that their study suggested that the heterozygous state may give rise to such clinical manifestations more often than had previously been expected. Rosan et.al.[46] found 23% of 212 patients with cystic fibrosis who were found to have a positive family history for diabetes mellitus. Panzram & Holstein[49] studied the sweat electrolyte values in 169 diabetics with an average age of 43.5 years and found that 37 diabetics gave elevated levels, 23 of which exceeded 100 mEq/L. Similar data are available for the relationship between peptic ulcer and cystic fibrosis. Koch[15] found increased incidence of duodenal and gastric ulcers in relatives of patients with cystic fibrosis and feels that it was a definite relationship between the two diseases.

SWEAT ELECTROLYTES IN ADULTS:

One of the major problems in the study of cystic fibrosis in adults, as it occurs in its homozygous, heterozygous or possibly forme fruste, was first discussed by Anderson & Freeman[50] in 1960 when they pointed out that the normal variation of sweat sodium and chloride levels in adults and in persons of varying age from children into adolescence and adult life had not been clearly defined. The authors were critical of interpretation of small variations in these levels indicating abnormality in adults. Their results, using

mecholyl stimulation in 100 normal adults, showed a wide scatter with 34% being higher than 60 mEq/L and only 23% below 40 mEq/L for sodium. They concluded that at about puberty and in adult life the level of 60 mEq/L used as the upper limit of normality by many authors could no longer be considered the dividing line between the normal and abnormal levels. They found that although the increase was greatest at puberty, it did not progress further with aging. The review by Robinson & Robinson[51] suggested that in thermal sweat, sodium chloride is extremely variable and recorded levels from 100-148 mEq/L down to 5 mEq.L, reported by a group of authors. This raised considerable question about the work on heterozygous carriers.[17,12] This was re-emphasized by Freeman & Anderson[52] in 1961 and later Lobeck & Huebner[53], studying sweat collected by pilocarpine iontophoresis, compared results from 42 normal children and from 43 normal adults over the age of 20 and found that there was considerable variation with age. McKendrick[54] performed a very extensive evaluation of the concentration of sodium and chloride in the sweat of adult patients, as well as those with chronic bronchits. He concluded that throughout childhood there is a slight rise in sweat sodium concentration and up to the age of 15 years, levels greater than 60 mEq/L are rare. A more abrupt rise occurs during puberty with rising levels throughout life. He found in a sample of 110 normal subjects between the ages of 15 and 84 years a mean sweat sodium concentration of 45.9 mEq/L, with a standard deviation of $\pm$18.5 mEq/L, compared with 191 normal subjects age 0-19 years who had a mean of 26.1 mEq/L with a standard deviation of $\pm$12.4 mEq/L. He limited the value of the estimation of sweat

sodium in the diagnosis of cystic fibrosis to infants with levels over 50 mEq/L and older children with levels over 70 mEq/L. Levels in siblings and parents show an average difference of 10 mEq/L for most ages, an unlikely expression of the heterozygous state. Siegenthaler et.al.[55] reiterated McKendrick's[54] conclusions in a study using pilocarpine iontophoresis in which they found significant difference between the concentrations in the 212 normal male and female subjects, 143 of whom were between the ages of 20-25 and 69 over the age of 50. They found mean values increased from 32.6 mEq.L at ages 20-25 to 54.3 mEq/L over the age of 50. Anderson et.al.[56] state that sweat sodium and chloride levels above 60-65 mEq/L should not be allowed to decide the diagnosis unless other definite features of cystic fibrosis are present. They concluded that as yet, no one had recorded a large enough series of normal or disease adults to make a dogmatic statement with regard to the full range of sweat electrolytes that can be found in adult life, and that although 60-70 mEq/L is the accepted upper limit of normal children, there is good reason to feel that it may be higher in adults. Sibinga & Barbero[57] and deHaller et.al.[60] further emphasized the variation in sweat electrolyte composition associated with age, not only between children and adults, but in adults as age increases.

There can be little doubt that the criticisms raised by these authors create great questions about the validity of most of the data in the literature on sweat electrolytes. A great mass of data is available on the concentration of electrolytes in the sweat of adults, most of which is related to various stimuli and stresses. Studies of

the composition of sweat in "normal" adults (Table I) have been done in various laboratories with a wide range of values obtained. One problem associated with this sort of data is that the normality of the population tested is not often defined, and many authors include as normal so-called "controls", who may be hospitalized with a disease, not thought to influence sweat electrolyte composition. The populations are relatively small, the largest being that of Siegnethaler of 212 normals. Although the introduction of the pilocarpine test[70] has resulted in more uniformity in sweat collection techniques, the results quoted as normal for adults are collected from various body areas, including the forearm[54,59,53,66,50,68], the hand[65,62,63,64], as well as the back[33] and the "whole body"[71,15]. Furthermore, there are variations in stimuli, including a thermal stimulus[65,71,62,33,63,64,27,15], mecholyl injection[66,50,49] and pilocarpine iontophoresis.[68,53,83,54,36] The technique for collection and analysis has also been variable, many of which used filter paper[68,53,54,83,54,49] or gauze pad,[66,50,63,64,15] both of which have problems associated with electrolyte contaminations, elution, dilution and weighing, contributing to the error of the method. A review of the values obtained for sweat electrolytes, as well as the technique used in the study of the adult homozygous cystic fibrosis cases, (Table II) emphasize the fact that evaluation of the sweat data is difficult. In many reports[19,26,25,23,18] there is no mention of the sweat collection site, stimulus or analytical technique.

STUDIES OF THE COMPOSITION OF SW

TABLE I.

Author	Age Range (years)	Collectn Site	Stimulus For Sweat	Sweat Collectn Technique	SODIUM (MEQ/L) Mean	Range	Std Dev
Johnson et al[62]	"young men"	Arm & Hand	Thermal	Rubber glove			
Darling[63]	adults	Palm	Thermal	Gauze pad			
Conn[64]	adults	Hand& Forearm	Thermal	Electrolyte Free Cotton	42	15-60	
Robinson et al[65]	young men	Hand& Forearm	Treadmill	Rubber glove			
Anderson et al[66]	adults	Forearm	Mecholyl	Gauze pad	56.0	27-87	
Smoller et al[17]	adults	Back	Heating pad	Gauze pad	32	8.0-113.0	S.E= ±2.2
Koch et al[15]	21-28	Whole Body	Bag	Gauze pad		17-70	
Anderson et al[50]	20-70	Forearm	Mecholyl	Gauze pad		20-120	
McKendrick[54]	15-84	Forearm	Pilo.	Filter paper	45.9	0-120	18.5
Kulcyzcki et al[36]	adults	Forearm	Pilo.	Filter paper	32.7	17-60	
Huhnstock et al[67]	17-70				55.1	±28.9	
Lobeck[53]	over 20	Forearm	Pilo.	Filter paper	39		
DiSant'Agnese[27]	20-80		Thermal	Gauze pad			
Siegenthaler[68]	20-25	Forearm	Pilo.	Filter paper	32.6	12-80	
	over 50	Forearm	Pilo.	Filter paper	54.3	15-116	
Lobeck et al[59]	20-60M	Forearm	Pilo.	Filter paper	51.9		±21.1
	20-60F	Forearm	Pilo.	Filter paper	36.5		±18.7
Panzram et al[49]	17-70	Forearm	Mecholyl	Filter paper	32.8	8.6-65.7	
Lobeck[69]	26-37	Forearm	Pilo.	Filter paper	53	15.8-99.8	
Coltman*	20-69	Forearm	Pilo.	Cellulose Sponge			

*Present Study.

2

STUDIES OF THE COMPOSITION OF SWEAT IN NORMAL ADULTS.

TABLE I.

		SODIUM (MEQ/L)				CHLORIDE (MEQ/L)				POTASSIUM (MEQ/L)			
timulus For veat	Sweat Collectn Technique	Mean	Range	Std Dev	Sample Size	Mean	Range	Std Dev	Sample Size	Mean	Range	Std Dev	Sample Size
ermal	Rubber glove						20-112						
ermal	Gauze pad					31.0	4-100	18.6	86				
ermal	Electrolyte Free Cotton	42	15-60		40								
readmill	Rubber glove						10.0-64.0						
echolyl	Gauze pad	56.0	27-87		20	52	19-82		20				
eating ad	Gauze pad	32	8.0-113.0	S.E= ±2.2	34	18.7	6.1-76.3	S.E= ±2.2	34				
ag	Gauze pad		17-70		32		16-60		32		7-20		32
echolyl	Gauze pad		20-120		100								
ilo.	Filter paper	45.9	0-120	18.5	110								
ilo.	Filter paper	32.7	17-60		25	26.6	9-46		32	9.4	2-23		25
		55.1	±28.9		183	51.3	±23.3		183				
ilo.	Filter paper	39			46								
ermal	Gauze pad						95% over 50		50				
ilo.	Filter paper	32.6	12-80		146								
ilo.	Filter paper	54.3	15-116		69								
ilo.	Filter paper	51.9		±21.1	33					7.5		±1.6	33
ilo.	Filter paper	36.5		±18.7	26					10.0		±2.1	26
acholyl	Filter paper	32.8	8.6-65.7		28	28.8	6.5-58.3		28				
ilo.	Filter paper	53	15.8-99.8		17	41.8	14.4-79.4		17	10.1	7.5-13.8		17
ilo.	Cellulose Sponge					27.3	6.7-75.2	±12.7	282				

REPORTS OF CYSTIC FIBROSIS IN ADULTS.

AUTHOR	NUMBER CASES	SWEAT VALUES IN mEq/L		METHOD OF SWEAT COLLECTION
		NA	CL	
Caldwell[28]	1	90	65	Loose fitting rubber glove overnight.
Brown et.al.[26]	1	155	135	Trunk heated and sweat collected from neck.
Frazier et.al.[23]	1	—	98-122	
Koch[31]	32	32-110	30-92	Whole body sweat.
Marks et.al.[24]	1	85	71	Mecholyl and gauze pad.
DiSant'Agnese[27] et.al.	1	107-119	76-10[illegible]	Bag and cotton gauze.
Ball[25]	1	101	102	———
Cece et.al.[30]	1	180.8	162	Rubber glove and thermal stimulus.
Alton[29]	1	65	65	———
Mertz et.al.[19]	1	139	90.9	———
Polgar[18]	4	74-110	—	———

TABLE II.

VARIABLES IN THE COLLECTION OF SWEAT:

There is a vast literature going as far back as the beginning of the century on the composition of sweat under various circumstances. Johnson et.al.[72], in 1944 studied the variables influencing the concentration of chloride in sweat and having reviewed the literature, found there was completed agreement by all workers on fours items, (1) the concentration of chloride tends to increase as work is prolonged (2) it varies between individuals (3) it varies with different regions of the body (4) it varies inversely as the supply of the drinking water. These factors account for some the wide range of values reported by Robinson & Robinson.[51] One of the major variables in the problem of sweat testing relates to the site of collection.[65, 71,73,74,75,76,77,78,79,80,81] Lobitz & Osterberg[76] state that palmar sweat concentrates chloride to a greater degree than had been previously appreciated. von Heyningen & Weiner[77] noted that the concentration of sweat in arm-bag sweat is always greater than that of total body sweat. Lobtiz[81], in an extensive review, agreed that palmar sweat tends to be more concentrated than that from other areas of the body.

There is considerable variation in the concentration of electrolytes, relative to the stimulus which is applied. There is a specific relationship between the skin temperature and electrolyte concentration.[62,65,71] The data obtained by the intradermal injection of mecholyl may be somewhat higher and ocassionally up to 20 mEq/L higher than that obtained by thermal sweat stimulation.[50,57]

The final consideration is the variability in the technique of

collection of the sweat. Collecting sweat from under a vapor impermeable barrier[81] will result in a decreased rate of potassium excretion. The technique of collecting whole sweat in bags and tubes allows for evaporation. The use of cotton gauze pad[27,17,66,63,64,15,50] or filter paper[68,53,54,38,49] involves the problems of weighing, elution and dilution which create error in analysis. Duboski[82] and Peterson[84] have developed techniques for obtaining pure sweat for analysis by extraction from cotton gauze or cellulose sponge, thus avoiding some of the errors associated with other techniques.

In conclusion, considerable doubt has been cast on the validity of the sweat test as used in the study of adult disease because of the many variables encountered related to age, site of collection, stimuli, method of collection and analytical technique. Although a large literature exists on the values for "normal" adult sweat electrolyte composition, because of these variables, one is unable to compare one group of data with another.

It was with this in mind that the author undertook to develop a relatively simple and reliable technique for sweat collection and apply it to the study of a large population of "normal" adult individuals.

MATERIALS AND METHODS.

The Population:

In an attempt to select a population which would approach normality as closely as possible, hospitalized patients were excluded. All persons were ambulatory and had been found to be "normal" on the

basis of a relatively recent complete physical examination and chest x-ray. In order to further ascertain their normality, a fairly extensive questionnaire was administered at the time of sweat collection (Appendix #1). All affirmative responses were further evaluated and if significant deviation from normal was ascertained, the individuals were excluded from the study.

The population tested included male and female employees of North American Aviation, Inc., Columbus, Ohio; male and female employees of the Southeastern Ohio Tuberculosis Hospital, Nelsonville, Ohio; male and female employees of the Ohio Tuberculosis Hospital, Columbus, Ohio; male inmates of the Honor Dormitory at the Ohio Penitentiary and selected ambulatory volunteers, consisting primarily of medical students and random employees of the Ohio State University Health Center. Although the population of normal people was derived from specific groups, there was no obvious selection for health reasons, and there were no significant variables which might necessarily influence the composition of their sweat. Sweat tests were performed during all seasons of the year, usually at the place of employment, and over a period between October 24, 1959 through April 1, 1963. Tests were performed by a variety of laboratory personnel, who adhered strictly to a technique devised by the author, using carefully controlled time intervals of sweat stimulation and collection and closely controlled analytical technique.

Data obtained was recorded on a file-catalog card (Appendix #2) and all analytical calculations were performed on an analysis form (Appendix #3). A data code was prepared (see Appendix #4) and thirty-

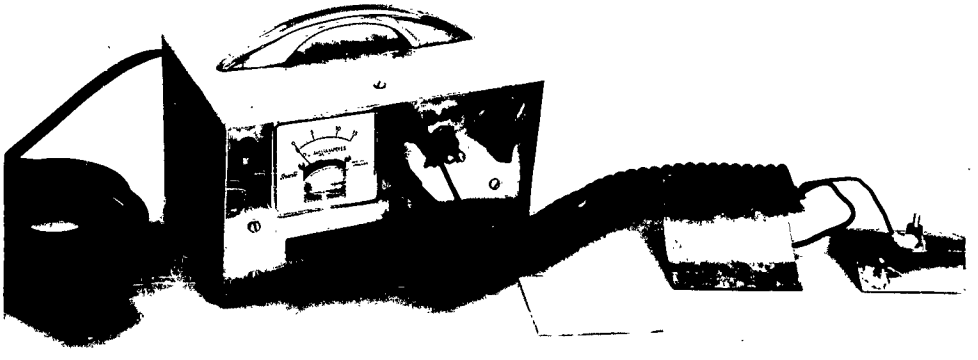
two items of relevant information were then recorded in code on an IBM data sheet (see Appendix #5); subsequently key punched onto IBM cards and processed.

SWEAT COLLECTION:

All samples of sweat recorded in this report were collected, using a technique reported by Coltman & Atwell[83] consisting of a combination of two previously published methods, the pilocarpine iontophoresis method of Gibson & Cook[70] and the cellulose sponge method for collecting sweat devised by Peterson[84]. A solution of pilocarpine nitrate is caused to pass into the sub-cutaneous tissues of the forearm over a period of 15 minutes by the application of a direct current of approximately 4 mamps. The presence of this compound results in localized sweating by the area into which the pilocarpine is applied. The sweat is subsequently collected in a small piece of cellulose sponge over a thirty-minute period of time. Pure sweat is then expressed from the sponge, using a chemically clean syringe, and directly analyzed for sodium, potassium and chloride.

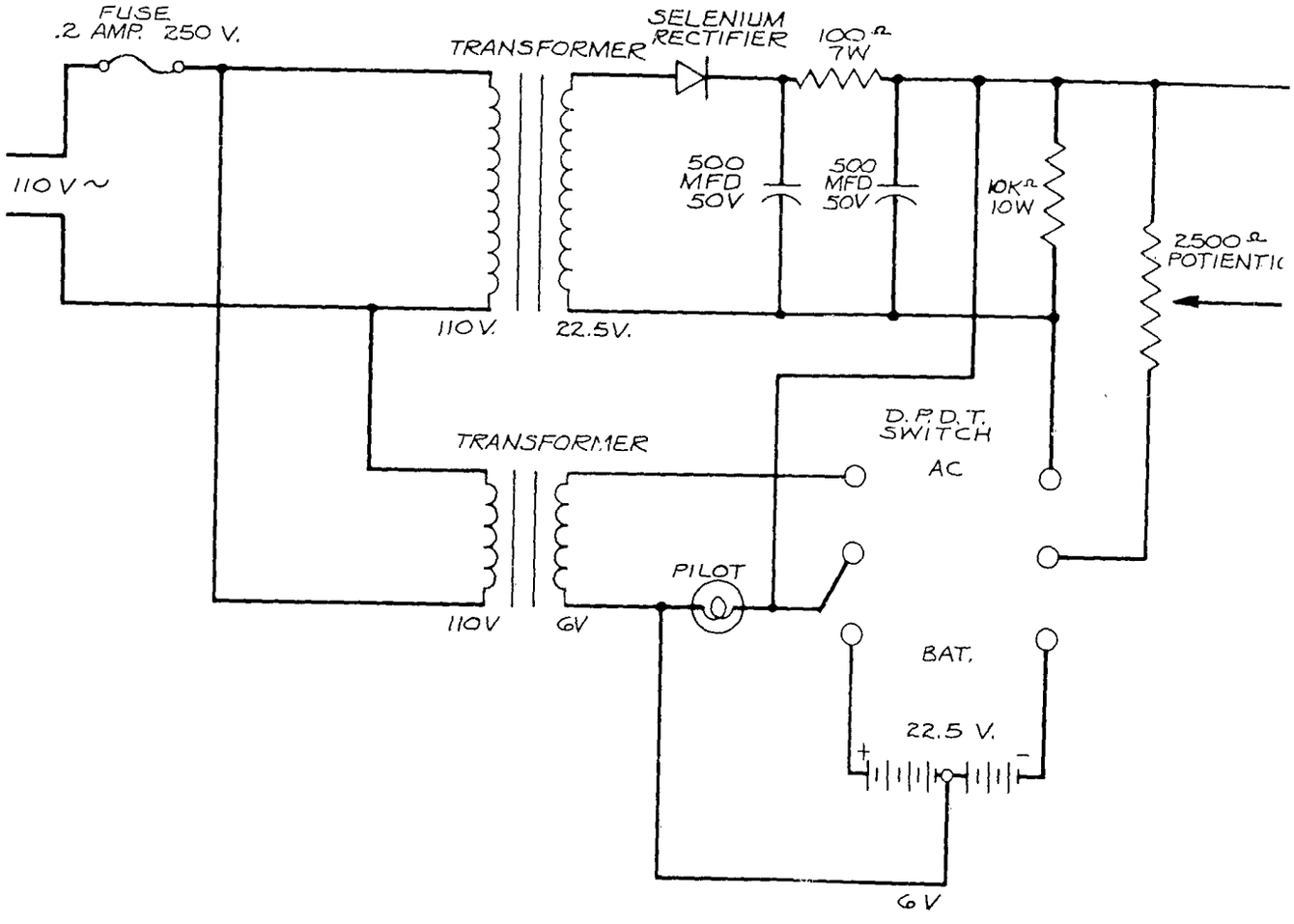
A modification of the original iontophoresis apparatus devised by Gibson & Cook[70] was built (Fig. 1 & 2). Current is derived from either a battery source or 110 volts AC power source by way of a selenium rectifier to provide direct current. Curved brass electrodes, measuring 3x3 inch in size were used as described in the alternative procedure of Gibson & Cook.[70]

An ordinary 4x4 inch cotton gauze pad is soaked in 4 ml of two-tenths per cent pilocarpine nitrate solution, and then applied to the positive electrode. A similar amount of 0.07 normal sodium bicarbonate



MODIFIED IONTOPHORESIS APPARATUS

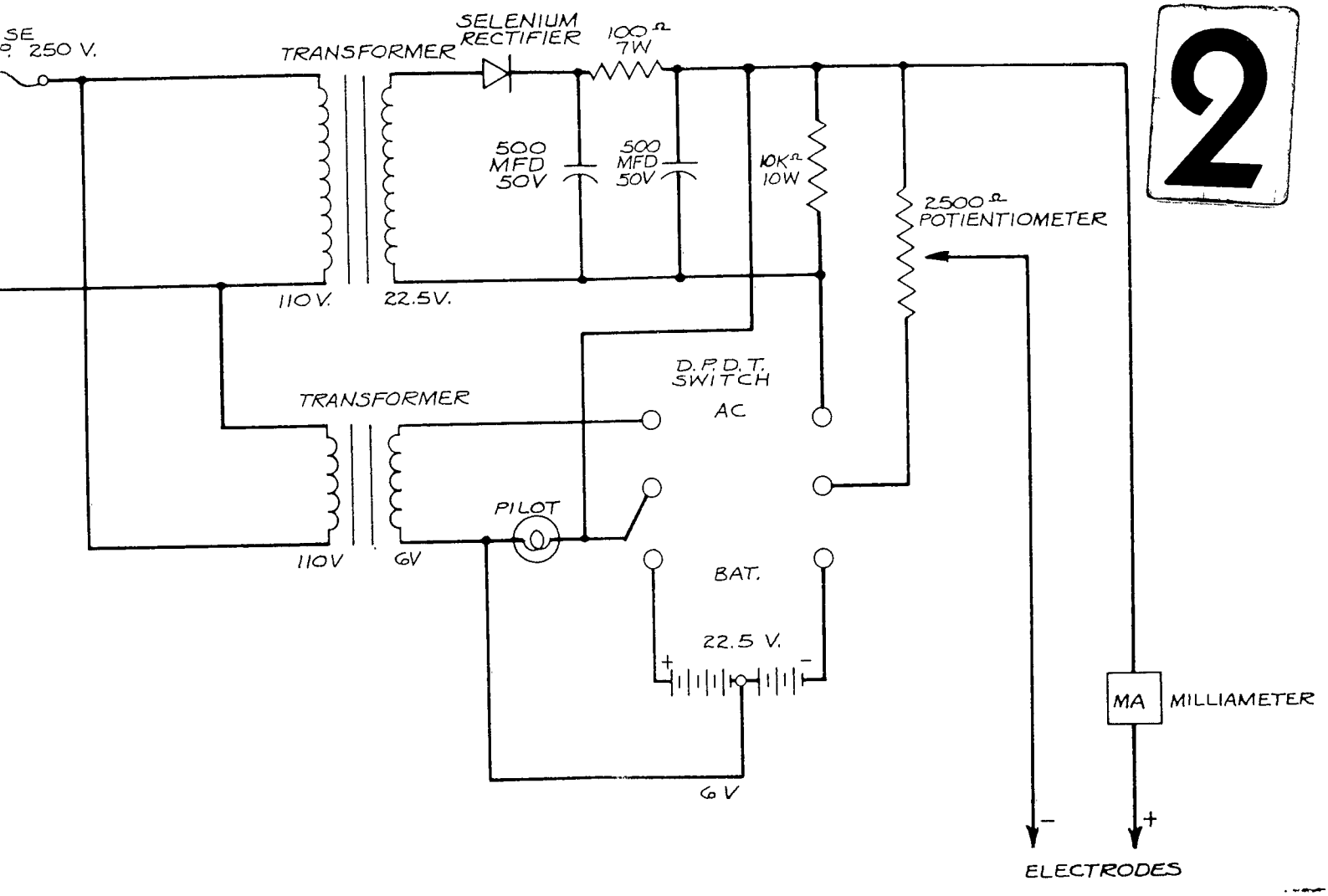
F I G U R E 1



IONTOPHORESIS
APPARATUS

WIRING DIAGRAM OF
MODIFIED IONTOPHORESIS APPARATUS

F I G U R E 2



ONTOPHORESIS APPARATUS

WIRING DIAGRAM OF
MODIFIED IONTOPHORESIS APPARATUS

FIGURE 2

solution is applied to a second gauze pad, covering the negative electrode. After scrubbing the forearm with double distilled water, the electrodes are mounted (Fig. #3) with the positive electrode covering the mid-volar aspect of the forearm, the negative electrode opposite and secured in place with rubber electrode straps. Care is taken to be sure that the electrodes have been completely covered with gauze to avoid contact with skin, which would result in a burn. With the electrodes in place the current is gradually raised to 4 mamps at which it is maintained, with periodic adjustments, for 15 minutes. Occasional parethesia can be alleviated by changing the tension on the electrode straps.

At the end of the 15-minute iontophoresis period the electrodes and gauze pads are removed; the forearm again scrubbed to a mild erythema in double-distilled deionized water and a cellulose collection sponge* (see Fig. #4) applied to the area beneath the positive electrode. The entire sponge is covered with saran wrap and held in place by adhesive tape. The saran wrap provides a vapor barrier under which sweat is collected over a period of 30 minutes. The sponge is then removed; used to mop up any free droplets of sweat, and placed in a 5 ml chemically cleaned syringe. Compression of the plunger yields pure sweat for analysis. An average of 0.164 ml of sweat remains in the sponge following complete extraction.

*The collection sponges were manufactured to our specifications by the American Sponge & Chamois Company, Long Island 1, New York and measure 2 5/8" x 2 3/4" by 1 mm in size.

IONTOPHORESIS ELECTRODES IN PLACE

F I G U R E 3

CELLULOSE SPONGE IN PLACE

F I G U R E 4

ANALYTICAL METHODS:

Sodium and potassium determinations are performed on an Advanced Flame Photometer, Model No. 11-B, made by the ADVANCED INSTRUMENTS, INC., Newton Highland, Massachusetts. The photometer has an internal lithium standard which is added in concentration of 300 parts per million to each unknown solution. A series of standard solutions are used, each containing concentrations of sodium, potassium and lithium ranging from sodium 0.10 mEq/L, potassium 0.25 mEq/L, lithium 43.2 mEq/L (300 parts per million) to sodium 1.60 mEq/L, potassium 0.40 mEq/L, lithium 43.2 mEq/L (300 parts per million). 0.100 ml of sweat is diluted to 10 ml with 303 parts per million of lithium solution, thereby bringing the lithium concentration to 300 parts per million. The sweat sample is read on the flame photometer and bracketed above and below with standard solutions of known concentrations and calibrated according to the curve of the standard reading. Quality control of the flame photometer is maintained twice weekly by analysis of a standard solution (Labtrol) containing 5.6 mEq/L of potassium and 51 mEq/L of sodium. When significant deviations from this result are obtained, new standard solutions are made up. A plot of the quality control data (Fig. 5 & 6) results in a standard deviation from the mean of twenty-one potassium determinations of $\pm$ 0.09 mEq/L and a standard deviation from the mean of twenty-eight sodium determinations of $\pm$ 0.76 mEq/L. Both of these results are within acceptable ranges of experimental error at these concentrations.

Chloride is analyzed by a BECKMAN-SPINCO, Model No. 153 microtitrator, using the ultra-micro modification of the technique of Schales

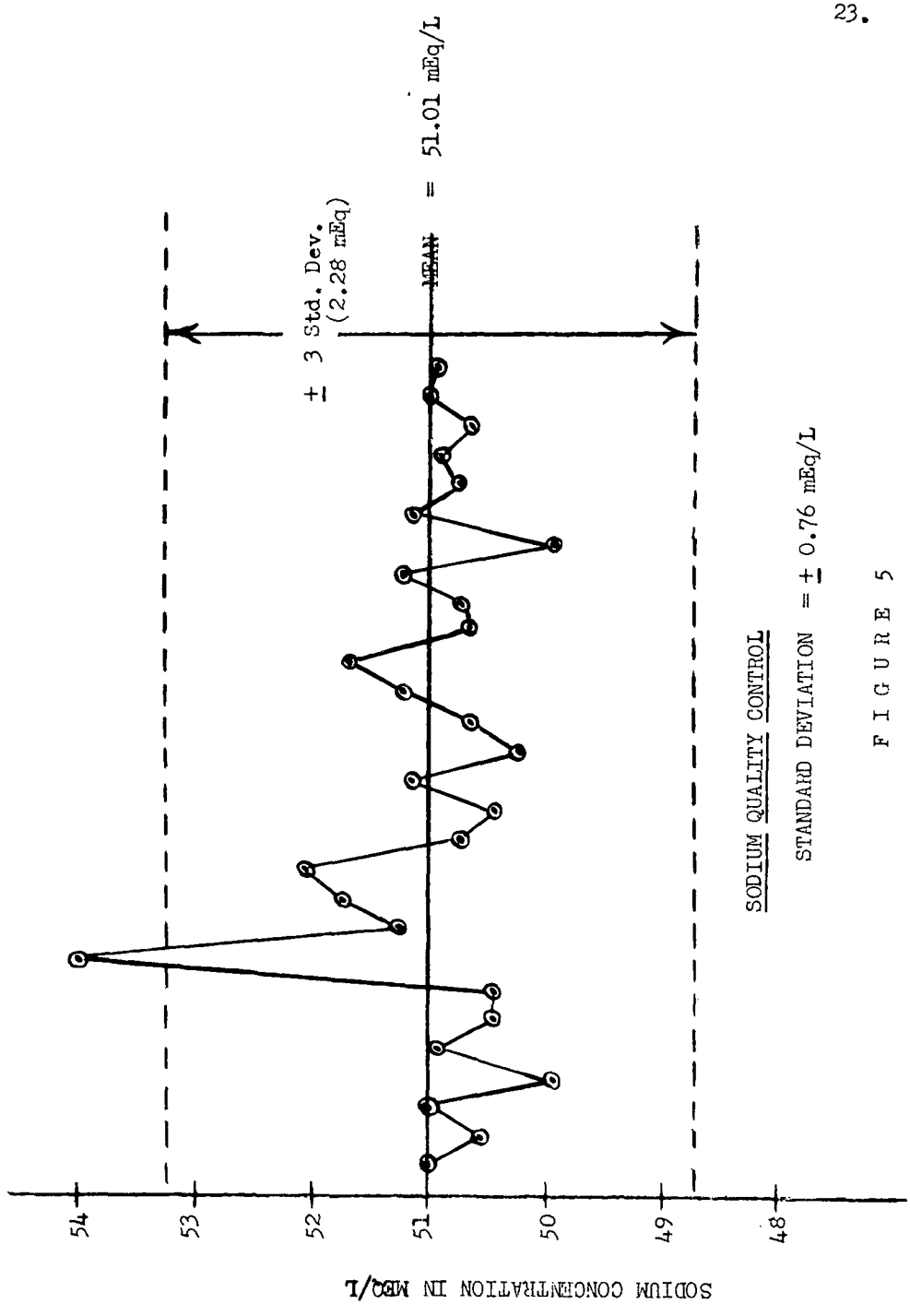

± 3 Std. Dev.
(2.28 mEq)
MEAN = 51.01 mEq/L
SODIUM QUALITY CONTROL
STANDARD DEVIATION = ± 0.76 mEq/L
SODIUM CONCENTRATION IN MEQ/L
54
53
52
51
50
49
48

FIGURE 5

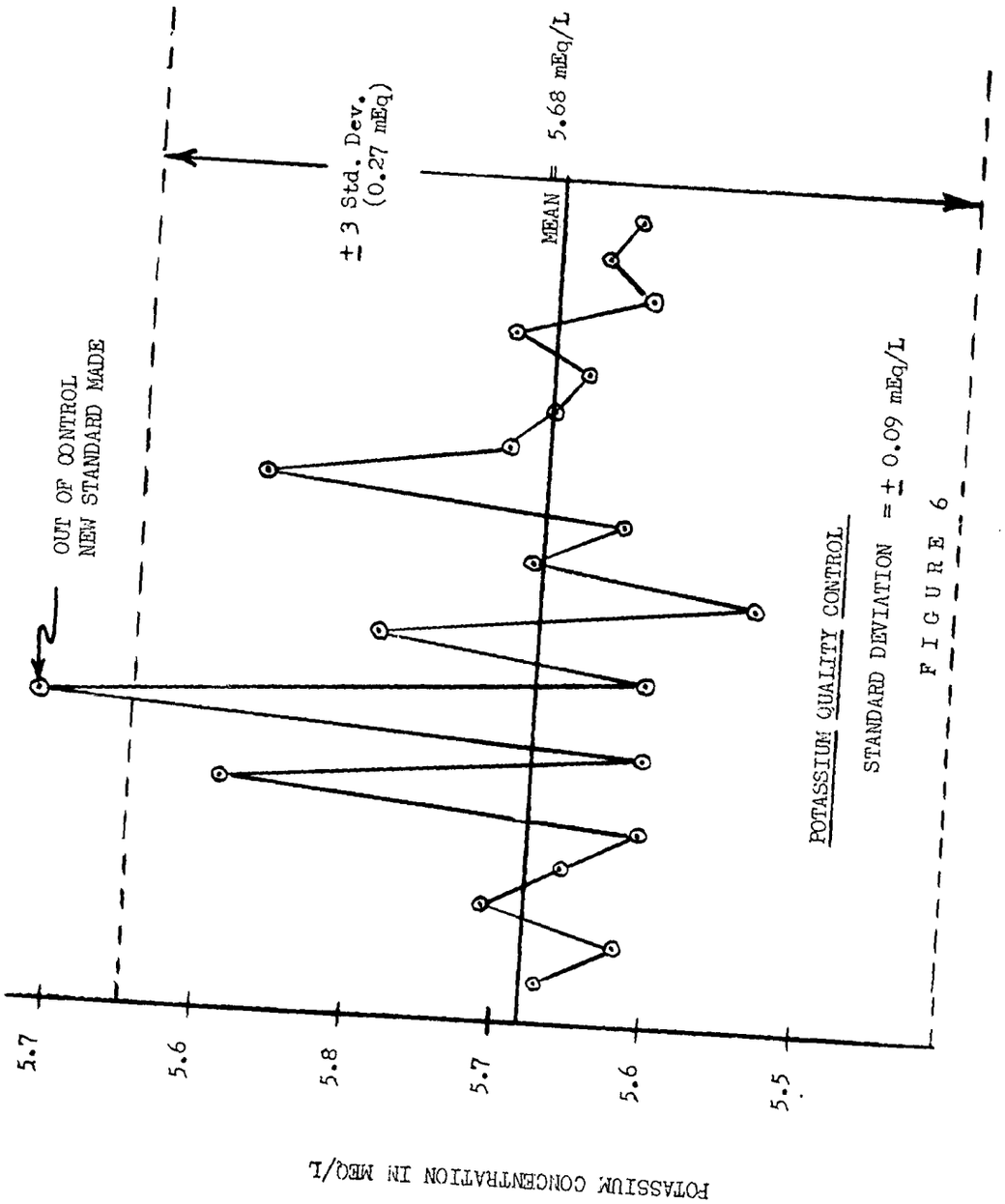

POTASSIUM CONCENTRATION IN MEQ/L
5.7
5.6
5.8
5.7
5.6
5.5
OUT OF CONTROL
NEW STANDARD MADE
± 3 Std. Dev.
(0.27 mEq)
MEAN = 5.68 mEq/L
POTASSIUM QUALITY CONTROL
STANDARD DEVIATION = ± 0.09 mEq/L

FIGURE 6

Schales[61] in which nitric acid, S-diphenylcarbazone and mercuric nitrate are used. 0.010 ml of sweat is titrated with an acid solution of mercuric nitrate in the presence of S-diphenylcarbazone indicator. The excess of mercuric ions form a blue-violet complex salt. A standard solution of known concentrations of NACL is titrated with each determination.

Quality control determinations of chloride (Fig. 7) are done at frequent intervals on a standard of 106 mEq/L. A standard deviation from the mean of twenty-one such determinations was ± 0.53 mEq/L, again within acceptable error.

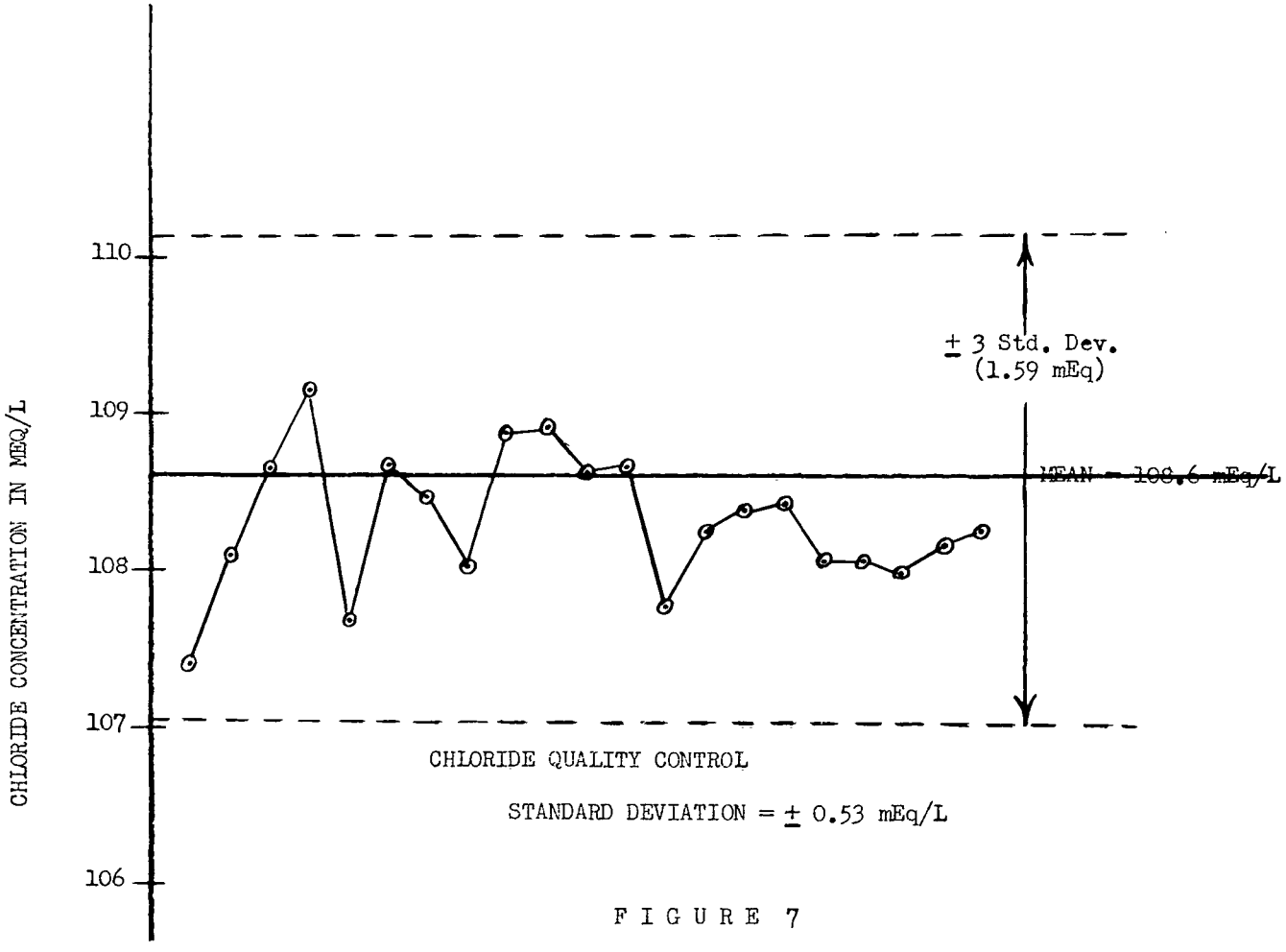
RESULTS:

Sponge Effect.

In an attempt to avoid the problems associated with weighing, elution and dilution in the analysis of sweat collected by established techniques, Peterson[84] used a cellulose sponge for sweat collection from which a sample of pure sweat is obtained. Peterson's original study[84] demonstrated that the washed* sponge did not materially affect the concentration of sodium and chloride of known solutions of various dilutions. He found an average per cent error for chloride determination of ± 2.38% and for sodium ± 1.93%, which he considered within the limits of experimental error.

During the course of this study, two shipments of one-thousand cellulose sponges were used. At the outset of the experiment a known

*Sponges were washed ten times in double-distilled deionized water.


CHLORIDE CONCENTRATION IN MEQ/L
110
109
108
107
106
± 3 Std. Dev.
(1.59 mEq)
MEAN = 108.6 mEq/L
CHLORIDE QUALITY CONTROL
STANDARD DEVIATION = ± 0.53 mEq/L

F I G U R E 7

solution of sodium chloride was prepared, containing 98.7 mEq/L of chloride and duplicate determinations were done before and after passage of this solution through five separate sponges, both washed and unwashed in an effort to determine the influence of the sponge on the concentration of sodium and chloride. This study (Table III) revealed that the error was less than one per cent in both the washed and unwashed sponges at this concentration of chloride and it was judged that the unwashed sponges were satsifactory for our study.

After the performance of 956 sweat tests, the second shipment of sponges was obtained and placed in use after more extensive testing. It would appear from these determinations on single sponges (Table IV & V), using ten concentrations, that washing the sponges resulted in a significant change in their adsorption characteristics. A similar test was done on the remaining few unwashed sponges from shipment #1 (Table VI), revealing a third and different set of data for actual and per cent error for both sodium and chloride determinations. Several attempts were made to encourage the data for the error induced by the sponges at various concentrations to adhere to a linear function, so as to devise a correction factor for electrolyte concentration which could be applied to each shipment. No specific function could be derived, and it was concluded that because single sponges were used at each concentration that sponge-to-sponge variation was too great to achieve this.

In order to evaluate the sponge effect on a fluid more closely resembling sweat, a solution was prepared, containing sodium and potassium chloride in concentrations of 30 mEq/L of sodium and 5

TEST OF SPONGE INFLUENCE

SHIPMENT #1

SODIUM CHLORIDE TEST SOLUTION

UNWASHED CHLORIDE*				WASHED CHLORIDE*			
Test Solution Conc. (mEq/L)		Actual Error	Per cent Error	Test Solution Conc. (mEq/L)		Actual Error	Per cent Error
PRE SPONGE	POST SPONGE			PRE SPONGE	POST SPONGE		
98.7	99.5	+0.8	+0.8%	98.7	99.5	+0.8	+0.8%

TABLE III

*Average of two determinations on five sponges.

TEST OF SPONGE INFLUENCE

SHIPMENT #2 (UNWASHED)

SODIUM CHLORIDE TEST SOLUTION

SODIUM*

Soln. #	Test Solution Conc. (mEq/L) PRE SPONGE	POST SPONGE	Actual Error	Per cent Error
1	11.64	14.14	+2.50	+21.45
2	25.37	25.38	0.00	0.00
3	38.64	38.22	-0.42	- 1.08
4	51.31	50.38	-0.92	- 1.79
5	66.35	62.32	-4.03	- 6.08
6	78.83	75.14	-3.69	- 4.69
7	92.72	86.46	-6.26	- 6.76
8	103.34	97.55	-5.79	- 5.60
9	117.14	113.24	-3.90	- 3.33
10	130.19	123.18	-7.01	- 5.38
AVERAGE ACTUAL ERROR			-2.95	
AVERAGE PER CENT ERROR				- 1.33%

CHLORIDE**

Soln. #	Test Solution Conc. (mEq/L) PRE SPONGE	POST SPONGE	Actual Error	Per cent Error
1	10.45	16.03	+5.58	+53.4
2	22.01	25.59	+2.68	+11.70
3	37.45	41.90	+4.45	+11.88
4	49.52	52.48	+2.96	+ 5.98
5	62.75	63.10	+0.35	+ 0.56
6	78.55	80.69	+2.14	+ 2.73
7	90.55	89.80	-0.75	- 0.83
8	102.31	101.18	-1.13	- 1.10
9	117.77	114.90	-2.87	- 2.92
10	128.61	125.86	-2.75	- 2.14
AVERAGE ACTUAL ERROR			+1.07	
AVERAGE PER CENT ERROR				+ 8.03%

TABLE IV

*Average of five determinations.
**Average of two determinations.

TEST OF SPONGE INFLUENCE

SHIPMENT #2 (WASHED)

SODIUM CHLORIDE TEST SOLUTION

SODIUM*

Soln #	Test Solution Conc. (mEq/L) PRE SPONGE	POST SPONGE	Actual Error	Per cent Error
1	11.64	10.00	-1.64	-14.08
2	25.37	22.43	-2.94	-12.78
3	38.64	36.05	-2.59	- 6.70
4	51.31	49.06	-2.25	- 4.39
5	66.35	61.69	-4.66	- 7.02
6	78.83	76.45	-2.38	- 2.99
7	92.72	89.70	-3.02	- 3.26
8	103.34	100.37	-2.97	- 2.87
9	117.14	114.11	-3.03	- 2.59
10	130.19	128.92	-1.27	- 0.98
AVERAGE ACTUAL ERROR			-2.6	
AVERAGE PER CENT ERROR				- 5.76%

CHLORIDE**

Soln #	Test Solution Conc. (mEq/L) PRE SPONGE	POST SPONGE	Actual Error	Per cent Error
1	10.45	10.72	+0.27	+2.58
2	22.91	23.12	+0.21	+0.9
3	37.45	37.70	+0.25	+0.67
4	49.52	51.15	+1.63	+3.29
5	62.75	63.10	+0.35	+0.56
6	78.55	79.39	+0.84	+1.07
7	90.55	90.57	+0.02	+0.00
8	102.31	103.79	+1.48	+1.44
9	117.77	118.10	+0.33	+0.28
10	128.61	129.12	+0.51	+0.40
AVERAGE ACTUAL ERROR			+0.58	
AVERAGE PER CENT ERROR				+1.12%

TABLE V

*Average of five determinations.
**Average of two determinations.

TEST OF SPONGE INFLUENCE

SHIPMENT #1 (UNWASHED)

SODIUM CHLORIDE TEST SOLUTION

SODIUM*

Soln #	Test Solution Conc. (mEq/L) PRE SPONGE	POST SPONGE	Actual Error	Per cent Error
1	12.21	12.64	+0.43	+3.52
2	25.98	25.75	-0.23	-0.88
3	37.66	36.57	-1.09	-2.89
4	51.28	51.12	-0.16	-0.31
5	62.31	63.87	+1.56	+2.50
6	77.57	76.16	-1.41	-1.82
7	91.46	90.61	-0.85	-0.93
8	105.86	101.40	-4.46	-4.22
9	117.89	115.21	-2.68	-2.25
10	135.34	131.26	-4.08	-3.01
AVERAGE ACTUAL ERROR			-1.38	
AVERAGE PER CENT ERROR				-0.81%

CHLORIDE**

Soln #	Test Solution Conc. (mEq/L) PRE SPONGE	POST SPONGE	Actual Error	Per cent Error
1	10.45	12.78	+2.33	+22.3
2	23.60	27.20	+3.60	+15.2
3	36.10	39.80	+3.70	+10.2
4	49.56	51.20	+1.64	+ 3.31
5	62.00	64.00	+2.00	+ 3.23
6	75.00	76.90	+1.90	+ 2.54
7	88.50	90.90	+2.40	+ 2.71
8	101.20	102.80	+1.60	+ 1.58
9	114.50	115.00	+0.50	+ 0.43
10	128.20	130.00	+1.8	+ 1.4
AVERAGE ACTUAL ERROR			+2.08	
AVERAGE PER CENT ERROR				+ 6.83%

TABLE VI

*Average of five determinations.
**Average of two determinations.

mEq/L of potassium. Duplicate determinations of sodium and potassium were made prior to and following passage of this solution through five spearate sponges, both washed and unwashed (Table VII). It should be noted that the average actual error for sodium is +0.26 mEq/L in the unwashed and -1.34 mEq/L in the washed sponges, both of which were less than the errors obtained when using pure sodium-chloride solution.

In a further extension of this study, frozen sweat samples were thawed, pooled and the previously described study performed on both pure sweat and a 1:1 dilution of sweat with double-distilled deionized water. These determinations (Table VIII & IX) resulted in further diminution in the average actual error and the average per cent error.

A test of the reproducibility of the sponge was done, using the potassium-chloride test solution. Ten sponges were soaked in a test solution containing a concentration of 4.74 mEq/L of potassium and the average actual error was found to be -0.68 mEq/L with a standard deviation of $\pm$ 0.098 mEq/L (Table X). Finally, determinations were done on the influence of the third shipment of sponges on potassium concentration and an average actual error of -0.35 mEq/L on unwashed sponges, -0.16 mEq/L on washed sponges were obtained. (Table VIII & IX).

The sponges in shipment #1 were used unwashed and those in shipments #2 and #3 have been used after washing. It can be concluded from the foregoing data that the influence of the sponge on a solution of pure sodium chloride is within $\pm$ 2.0 mEq/L at all concentrations. Furthermore, in studies of the influence of the sponge on the concentration of multiple ionic solution such as sweat, this vari-

TEST OF SPONGE INFLUENCE

SHIPMENT #3

SODIUM AND POTASSIUM CHLORIDE TEST SOLUTION

UNWASHED*

SODIUM			POTASSIUM		
Test Solution Conc. (mEq/L)		Actual	Test Solution Conc. (mEq/L)		Actual
PRE SPONGE	POST SPONGE	Error	PRE SPONGE	POST SPONGE	Error
30.90	31.16	+0.26	5.15	4.50	-0.65
30.90	31.50	+0.60	5.15	4.64	-0.51
30.90	31.03	+0.13	5.15	4.62	-0.53
30.90	30.84	-0.06	5.15	4.54	-0.61
30.90	31.15	+0.25	5.15	4.50	-0.65
AVERAGE ACTUAL ERROR		+0.26	AVERAGE ACTUAL ERROR		-0.54
AVERAGE PER CENT ERROR		+0.84	AVERAGE PER CENT ERROR		±10.40

WASHED*

SODIUM					
Test Solution Conc. (mEq/L)		Actual	Test Solution Conc. (mEq/L)		Actual
PRE SPONGE	POST SPONGE	Error	PRE SPONGE	POST SPONGE	Error
30.90	29.60	-1.30	5.15	4.54	-0.61
30.90	29.95	-0.95	5.15	4.74	-0.41
30.90	29.30	-1.60	5.15	4.58	-0.67
30.90	29.30	-1.60	5.15	4.64	-0.57
30.90	29.33	-1.57	5.15	4.70	-0.45
AVERAGE ACTUAL ERROR		-1.34	AVERAGE ACTUAL ERROR		-0.55
AVERAGE PER CENT ERROR		-4.3	AVERAGE PER CENT ERROR		±10.6%

TABLE VII

*One determination on each of five sponges.

TEST OF SPONGE INFLUENCE

SHIPMENT #3 (UNWASHED)

POOLED PURE SWEAT TEST SOLUTION

#	SODIUM* Test Solution Conc. (mEq/L) PRE SPONGE	POST SPONGE	Actual Error	Per cent Error	CHLORIDE* Test Solution Conc. (mEq/L) PRE SPONGE	POST SPONGE	Actual Error	Per cent Error
1	12.23	11.44	-0.79	-6.38%	12.11	12.72	+0.61	+5.03%
2	26.30	24.94	-1.36	-5.16%	24.02	24.85	+0.83	+3.34%
AVERAGE ACTUAL ERROR			-1.07		AVERAGE ACTUAL ERROR		+0.72	
AVERAGE PER CENT ERROR				-5.77%	AVERAGE PER CENT ERROR			+4.19%

#	POTASSIUM* Test Solution Conc. (mEq/L) PRE SPONGE	POST SPONGE	Actual Error	Per cent Error
1	3.32	3.13	-0.19	-5.75%
2	6.62	6.12	-0.50	-7.55%
AVERAGE ACTUAL ERROR			-0.35	
AVERAGE PER CENT ERROR				-6.65%

TABLE VIII

TEST OF SPONGE INFLUENCE

SHIPMENT #3 (WASHED)

POOLED PURE SWEAT TEST SOLUTION

#	SODIUM Test Solution Conc. (mEq/L) PRE SPONGE	POST SPONGE	Actual Error	Per cent Error	CHLORIDE Test Solution Conc. (mEq/L) PRE SPONGE	POST SPONGE	Actual Error	Per cent Error
1	12.23	11.57	-0.66	-5.39	12.11	12.72	+0.61	+4.79
2	26.30	25.57	-0.73	-2.77	24.02	24.45	+0.43	+1.78
AVERAGE ACTUAL ERROR			-0.69		AVERAGE ACTUAL ERROR		+0.52	
AVERAGE PER CENT ERROR				-4.08%	AVERAGE PER CENT ERROR			+3.29%

#	POTASSIUM Test Solution Conc. (mEq/L) PRE SPONGE	POST SPONGE	Actual Error	Per cent Error
1	3.32	3.15	-0.17	-5.72
2	6.62	6.47	-0.15	-2.26
AVERAGE ACTUAL ERROR			-0.16	
AVERAGE PER CENT ERROR				-3.99%

TABLE IX

TEST OF SPONGE INFLUENCE

SHIPMENT #3 (WASHED)

POTASSIUM CHLORIDE TEST SOLUTION

POTASSIUM*

Test Solution Conc. (mEq/L)		Actual
PRE SPONGE	POST SPONGE	Error
4.74	4.10	-0.64
4.74	3.92	-0.82
4.74	4.06	-0.68
4.74	4.07	-0.67
4.74	4.16	-0.58
4.74	4.15	-0.57
4.74	3.97	-0.81
4.74	4.13	-0.61
4.74	3.91	-0.83
AVERAGE ACTUAL ERROR		-0.68 mEq/L

TABLE X

*Standard Deviation = 0.098 mEq/L.

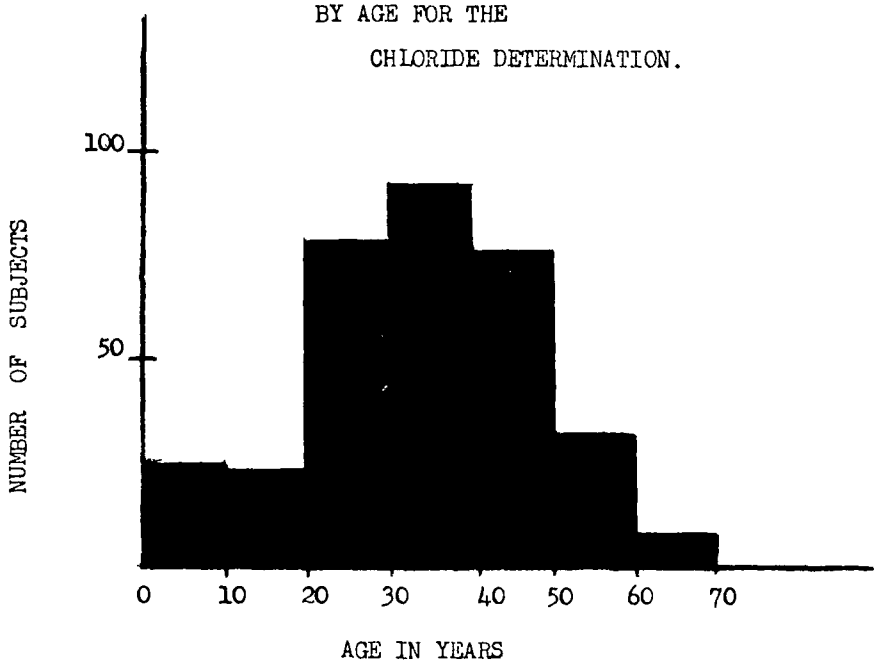
ation becomes less significant and falls within less than ± 1.0 mEq/L for all ions involved. The variation in absorption characteristics from sponge to sponge is relatively small. The error associated with the use of the washed or unwashed cellulose sponge to absorb pure sweat is at or below the error associated with other steps in the procedure and certainly less than that introduced during the multiple steps of weighing, elution and dilution of other techniques of sweat collection.

DATA ON "NORMALS"

In this experiment 355 sweat tests were performed on a group of normal persons, ranging in age from less than 3 months to 69 years. (Table XI & Fig. 8). One hundred eighty-six males and one hundred sixty-three females were studied. A sample, varying in size from 0.10 mEq/L to 2.01 ml. with an average of 0.39 ml (mean for male -0.51 ml. and mean for female -0.25 ml.) was obtained and was adequate for the analysis of chloride in 96.9% of tests. The frequency distributions of the values obtained for sodium, potassium and chloride show some skew to the right (Figures 9, 10, 11). The sample size, mean and range of the values obtained for sodium, potassium and chloride (Table XII) include all ages.

A study of the influence of sex on the concentration of sodium, potassium and chloride (Table XIII) in sweat reveals that the difference between the mean value of sodium and chloride concentration for males compared to females is not significant and has a P value of 0.32 and 0.84 respectively. The difference between the mean potassium concentration for males and females closely approaches, but does not

DISTRIBUTION OF SUBJECTS
BY AGE FOR THE
CHLORIDE DETERMINATION.



F I G U R E 8

AGE INFLUENCE ON ELECTROLYTES

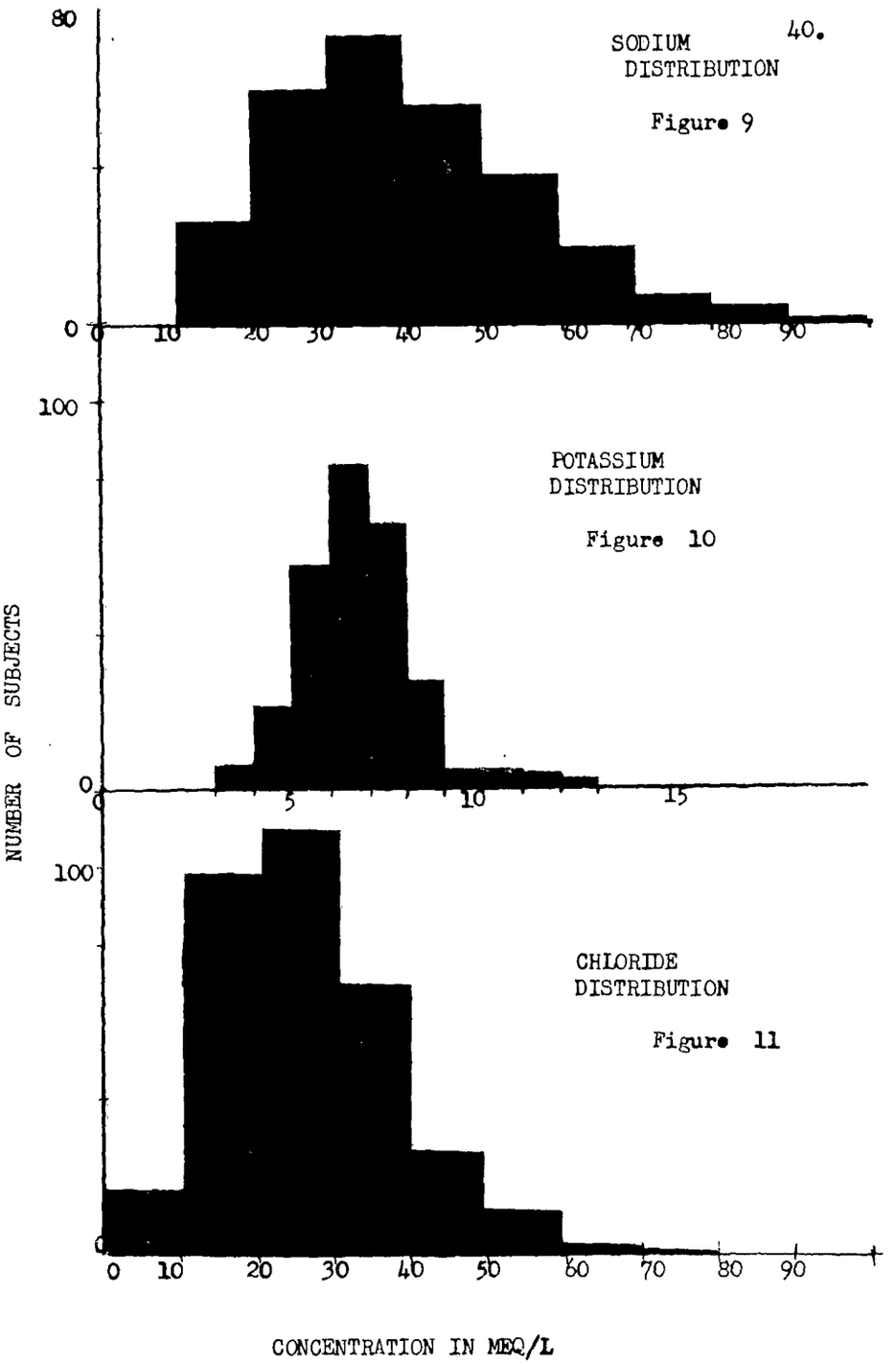
Age	Mean	Standard Deviation	Sample Size
Na:			
0-20	35.5	13.4	39
21-29	40.3	16.0	66
30-39	43.0	15.6	79
40-49	39.8	16.0	66
50-59	39.8	15.1	28
60-69	39.5	9.5	6
K:			
0-20	6.9	1.2	36
21-29	6.8	1.6	66
30-39	6.5	1.5	77
40-49	6.7	1.3	66
50-59	6.6	1.3	27
60-69	5.9	0.7	6
Cl:			
0-20	25.0	9.9	48
21-29	27.5	12.3	78
30-39	28.2	13.5	91
40-49	26.7	12.1	75
50-59	25.0	12.2	31
60-69	29.6	12.7	1

TABLE XI

NORMAL DATA

	Mean	Range	Sample Size
SODIUM	40.1	13.0-91.9	290
POTASSIUM	6.5	3.2-12.1	283
CHLORIDE	26.9	6.7-75.2	338

TABLE XII



SODIUM DISTRIBUTION

Figure 9

POTASSIUM DISTRIBUTION

Figure 10

CHLORIDE DISTRIBUTION

Figure 11

SEX INFLUENCE

	MALES				FEMALES		
	P	MEAN	STD. DEV.	SAMPLE SIZE	MEAN	STD. DEV.	SAMPLE SIZE
SODIUM	.32	40.8	16.0	173	39.9	15.0	117
POTASSIUM	.05	6.5	1.5	171	6.9	1.6	112
CHLORIDE	.84	27.0	12.2	183	26.7	12.3	156

TABLE XIII

reach a level of statistical significance with a P value of 0.051.

The influence of the season of the year in which the study was done on the concentration (Table XIV) and the volume (Fig. 12) was studied. Tests performed between December and February were compared to those done during June through August. There is not a statistically significant difference between the mean potassium and chloride concentrations of those two periods of twime. The mean sodium concentration is higher during the winter months with a difference between the means

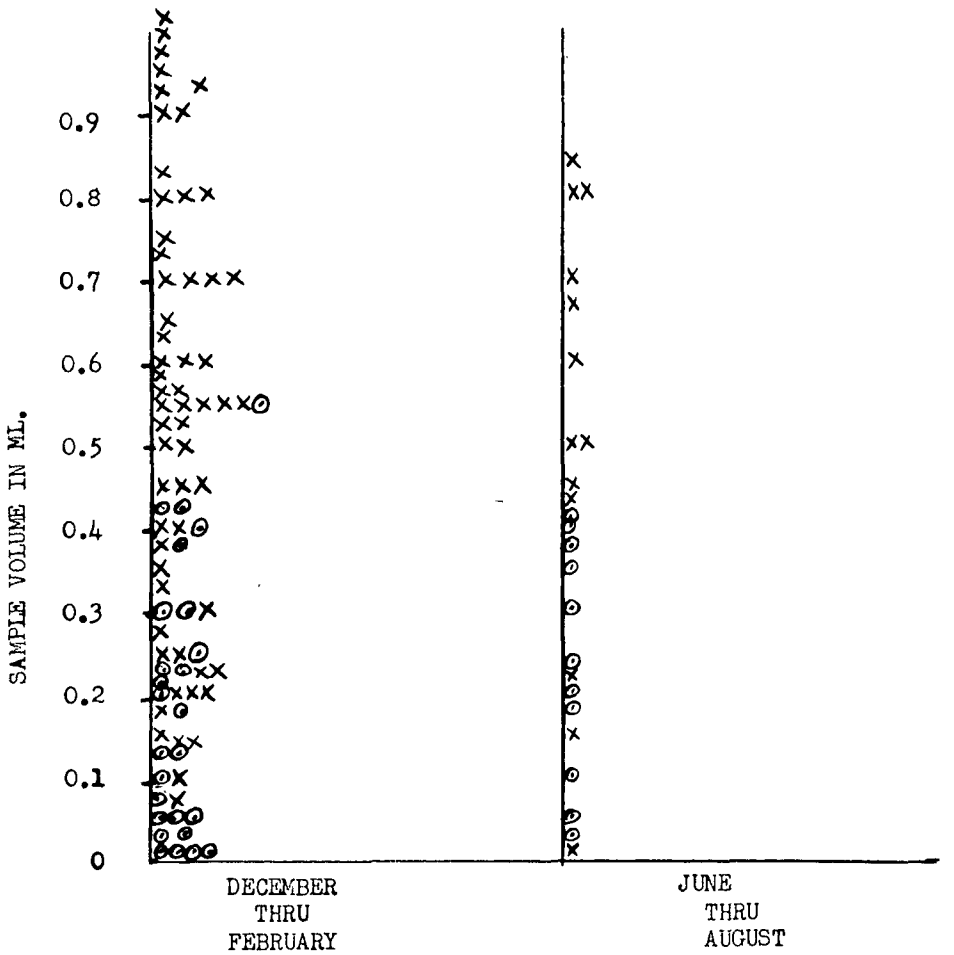
SEASONAL INFLUENCE

	DEC THRU FEB				JUNE THRU AUGUST		
	P	MEAN	STD. DEV.	SAMPLE SIZE	MEAN	STD. DEV.	SAMPLE SIZE
SODIUM	0.002	44.4	15.4	70	32.5	15.9	22
POTASSIUM	0.48	6.3	1.9	69	6.5	1.1	19
CHLORIDE	0.62	26.6	11.2	84	24.8	14.4	22

TABLE XIV

which would occur by chance less than 2 out of 1,000 times. The mean sample size collected during the winter months was larger, probably accounted for by the fact that more men (average sample size of 0.51

RELATIONSHIP OF SWEAT
"RATE" TO SEASON
X= MALE
⊙= FEMALE

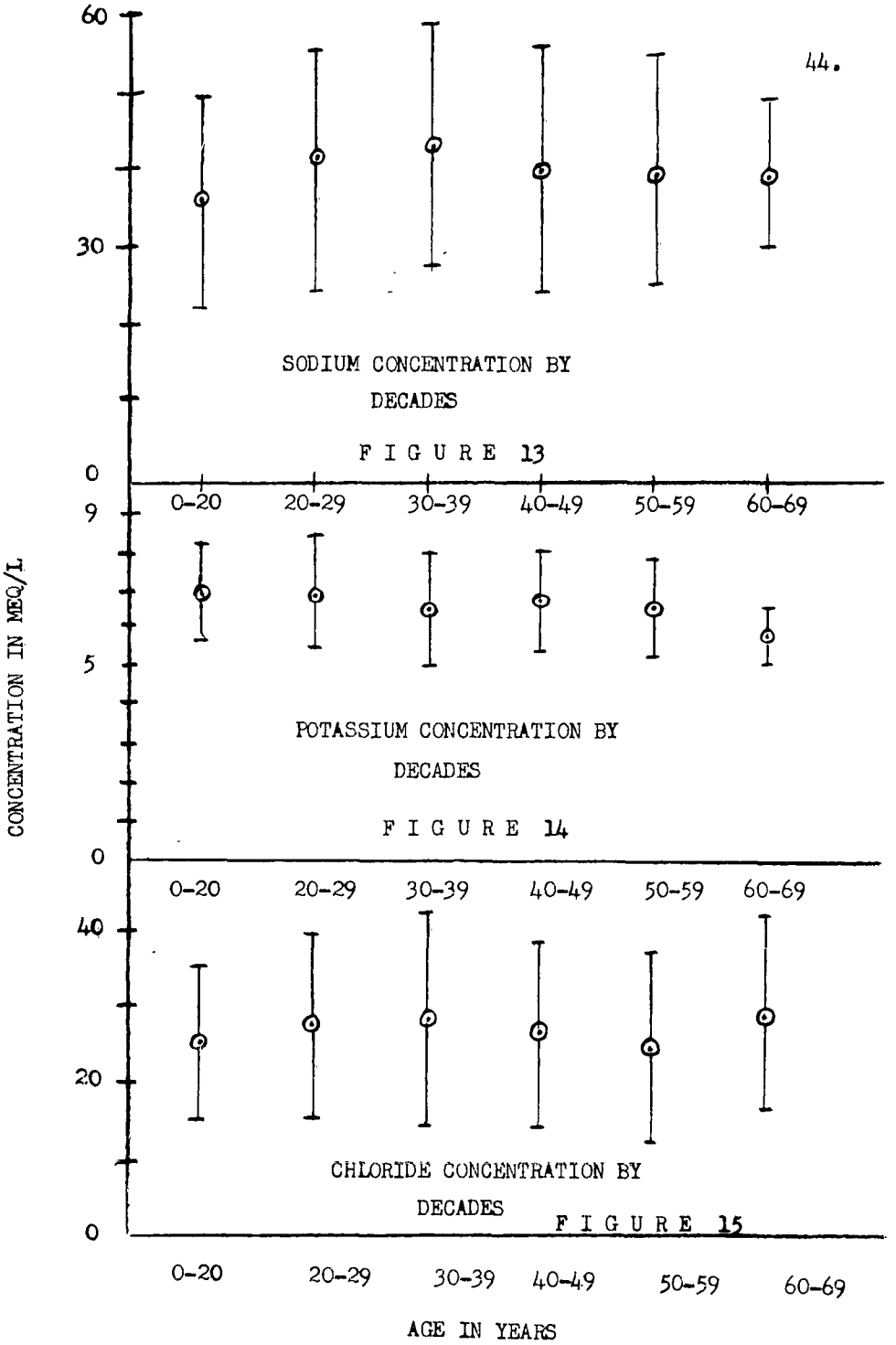


F I G U R E 12

ml) than women (average sample size of 0.25 ml) were tested. This fact does not account for the increased sodium concentration of sweat collected from December to February as there is no significant difference between the sodium concentration of sweat in males and females (Table XIII) or an increase in sodium concentration with increasing sample size (Fig. 16).

There are definite indications that the concentrations of sodium and chloride below the age of 20 are lower than those above (Table XI) (Figures 13-15). Above the age of 20 there is no consistent relationship of sodium or chloride to age. The potassium concentration, however, (Fig. 14) appears to show a definite trend toward diminution in concentration with increasing age, the mean concentration below 20 years, being 6.9 mEq/L and that between 60 and 69 being 5.9 mEq/L.

A consideration of the influence of sweat rate on the concentration of sodium, chloride and potassium is important and although we do not have specific information in regard to sweat rate, the volume of sweat expressed from each sponge was carefully measured. This represents a "sweat rate" insofar as the sponge in all instances was left in place for a total of 30 minutes, and all sweat collected during that period of time was absorbed onto the sponge. The average of 0.169 cc of sweat left in each sponge plus the volume expressed from it represents the volume of sweat produced during the 30-minute period of sponge application. This, in a sense, represents a "sweat rate". There is no significant relationship between the concentration of sodium, potassium and chloride and the "rate" at which sweating occurs under these circumstances (Fig. 16).



SODIUM CONCENTRATION BY DECADES

FIGURE 13

POTASSIUM CONCENTRATION BY DECADES

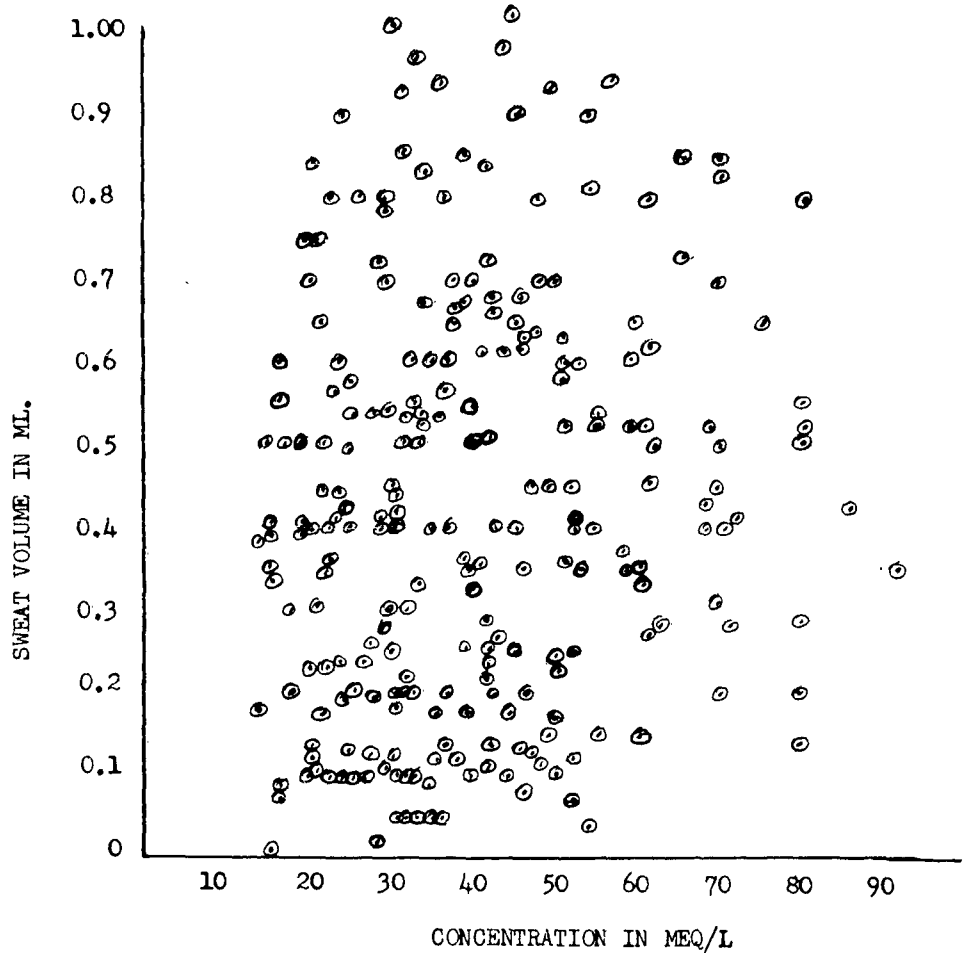
FIGURE 14

CHLORIDE CONCENTRATION BY DECADES

FIGURE 15

CONCENTRATION- VOLUME
RELATIONSHIP

SODIUM*



F I G U R E 16

*Potassium and Chloride Demonstrate Similar Relationships.

In conclusion, an attempt was made to determine the range of normal electrolyte composition of the sweat of normal adult males and females collected over a period of thirty minutes by a cellulose sponge, following stimulation by pilocarpine iontophoresis. The influence of the patient's sex and age; the seasons during which the sample was collected and the "rate of sweating" were considered. Because the concentration of electrolyte in the sweat of individuals below the age of 20 is lower than above that age, this group of patients must be excluded in consideration of the normal composition of sodium, potassium and chloride in adults. Analysis of the influence of sex reveals that the concentration of potassium in female sweat is somewhat higher than that in males. The concentration of sodium in samples collected during the winter months is statistically significantly higher than that during the summer months. The so-called "rate of sweating" appears to influence neither sodium, potassium nor chloride concentration. The concentration of chloride in the sweat of adults appears, therefore, to be the most reliable and invariable determinant, being influenced by sex, rate of sweating or season of collection.

DISCUSSION AND CONCLUSIONS:

Cystic fibrosis of the pancreas is a genetically determined disease of relatively high gene frequency[4,9,11] inherited as a Mendelian recessive trait.[3,4,11] The gene occurs in the adult population with a frequency of somewhat less than one in twenty.[11] It is probably maintained in the population in high frequency by a

slight heterozygotic selective advantage.[11,14]

The heterozygous form of the disease, as it occurs in adult carriers, is not uniformly recognizable by any presently available study although definite relationships to certain adult disease states have been recognized.[3,15,33,34,43,49]

Studies of sweat electrolytes in adults[15,17] have been criticized[52,54] because the electrolyte values quoted as being the upper limit of normal in children are not considered applicable to adults. The literature in criticism of this data, each use different techniques for sweat stimulation and collection, which in turn, differs from the methods used in the study under criticism (Table I). Furthermore, variations in results have been related to location of collection,[65, 71,73,74,75,76,77,78,79,80,81] stimulus of sweating[50,51,62,65,71] and collection technique.[33,81] A review of the data, presently available in the literature on the values of sweat electrolytes of normal adults, reveals that all of these variables are operative, thus limiting the value of this data when comparing them with one another.

A technique for the rapid collection of pure sweat, using pilocarpine iontophoresis and cellulose sponge for collection has been thoroughly evaluated and found to have a reliability well within the range of acceptable experimental error. Using this method, the electrolyte composition of a large population of normal children and adults was studied in an attempt to provide data on the normal values for adults.

The mean range for the sodium, potassium and chloride content were found for the population as a whole (Table XII). Statistical

analysis of this data reveals that: (1) the sweat potassium appears to be higher in women than in men as has been previously reported[53] (Table XIII), (2) the sweat sodium and chloride, but not potassium tend to be lower in normal persons below twenty years of age (Table XI), (3) there appears to be, in contrast to other reports, no uniform trend for sodium and chloride levels above the age of twenty, but the potassium level tends to decline somewhat with age, (4) the sweat sodium collected during the winter months is, for some unknown reason, significantly higher than during the summer months, (5) although men on an average provide a sweat sample twice as large as women, this does not seem to influence the electrolyte composition of the sweat, (6) the mean sweat potassium of 6.5 mEq/L with a range of 3.2-12.1 mEq/L and a standard deviation of 1.5-1.6 mEq/L covers a surprisingly narrow range, and should prove helpful in analysis of abnormal states.

It appears that, using this technique, the chloride content of "normal" adult sweat is that parameter least influenced by age, sex, season and sweat "rate". The value obtained for normal men and women (282 subjects) over the age of 20 years, using the technique of Coltman & Atwell,[83] a mean of 27.3 mEq/L with a range from 6.7 mEq/L to 75.2 mEq/L. The standard deviation is $\pm$ 12.7. Two standard deviations (essentially 95% of the observations) fall below 52.7 mEq/L, a level surprisingly close to that quoted by Smoller & Hsia[17] and DiSant'Agnese[27] in their original studies on adult heterozygotes. To date, this is the largest series of sweat tests reported on normal adult men and women.

Of further interest is the fact that the frequency distribution of the normal values (Fig. 9-11) for all electrolytes assumes the configuration of a nongausian distribution with a skew to the right. This suggests that we are dealing with a bimodal distribution, consisting of two populations within the "normals". This could be accounted for by a population of homozygous normals, being skewed to the right by a population of heterozygotes indistinguishable from normal, except by their higher sweat test. Evaluation of this hypothesis is in process and conclusion must await the study of the distribution of a large number of diseased adults in an attempt to depicit a trimodal population, the third group, being those adults with a homozygous condition.

SUMMARY:

An attempt has been made to review the genetics of cystic fibrosis of the pancreas and those points of particular interest to the internist have been emphasized. A simple, rapid technique for sweat collection has been presented, tested and applied to a large population of "normal" adults.

Test #____Date_____Age___Sex____Race_____HT._____WT._____

Name_____Hospital No._____Phone No._____
Last First Middle

Address_____Sweat Test No._____Analysis No._____

A. PAST MEDICAL HISTORY: (Elaborate on positives on back)

		Yes	No			Yes	No
1.	Measles			21.	Asthma		
2.	Mumps			22.	Wheezing		
3.	Varicella			23.	Pleurisy		
4.	Pertussis			24.	Short of breath		
5.	Scarlet Fever			25.	Bronchitis		
6.	Tuberculosis			26.	Chest Pain		
7.	Rheumatic Fever			27.	Flu		
8.	Diabetes			28.	Pneumonia		
9.	Hypertension			29.	Hemoptysis		
10.	Jaundice			30.	Kidney disease		
11.	Salt Craving			31.	Heart disease		
12.	Heat Stroke			32.	Thyroid disease		
13.	Chronic diarrhea			33.	Ulcers		
14.	Bulky Stools			34.	Pancreatitis		
15.	Foul smelling stools			35.	Liver disease		
16.	Salty sweat			36.	Allergies		
17.	Salivary Gland disease			37.	Sweat easily		
18.	Frequent chest colds			38.	Dry mouth		
19.	Chronic cough			39.	Painful breasts		
20.	Sinusitis			40.	Use of Cigarettes?		

B. FAMILY HISTORY:

		Yes	No
1.	Anyone in family have the above problems?		
2.	Other serious illness?		

		AGE	C.O.D./or PRESENT HEALTH
3.	Father		
4.	Mother		
5.	Siblings		
6.	Offspring		

C. PHYSICAL FINDINGS:

D. LABORATORY DATA:
Hb:
Hct:
WBC:

F. SWEAT ELECTROLYTES:
Time:
Volume:
NA:

Name_____ Hospital No._____ Phone No._____
Last First Middle

Address_____ Sweat Test No._____ Analysis No._____

A. PAST MEDICAL HISTORY: (Elaborate on positives on back)

		Yes	No			Yes	No
1.	Measles	—	—	21.	Asthma	—	—
2.	Mumps	—	—	22.	Wheezing	—	—
3.	Varicella	—	—	23.	Pleurisy	—	—
4.	Pertussis	—	—	24.	Short of breath	—	—
5.	Scarlet Fever	—	—	25.	Bronchitis	—	—
6.	Tuberculosis	—	—	26.	Chest Pain	—	—
7.	Rheumatic Fever	—	—	27.	Flu	—	—
8.	Diabetes	—	—	28.	Pneumonia	—	—
9.	Hypertension	—	—	29.	Hemoptysis	—	—
10.	Jaundice	—	—	30.	Kidney disease	—	—
11.	Salt Craving	—	—	31.	Heart disease	—	—
12.	Heat Stroke	—	—	32.	Thyroid disease	—	—
13.	Chronic diarrhea	—	—	33.	Ulcers	—	—
14.	Bulky Stools	—	—	34.	Pancreatitis	—	—
15.	Foul smelling stools	—	—	35.	Liver disease	—	—
16.	Salty sweat	—	—	36.	Allergies	—	—
17.	Salivary Gland disease	—	—	37.	Sweat easily	—	—
18.	Frequent chest colds	—	—	38.	Dry mouth	—	—
19.	Chronic cough	—	—	39.	Painful breasts	—	—
20.	Sinusitis	—	—	40.	Use of Cigarettes?	—	—

B. FAMILY HISTORY:

1. Anyone in family have the above problems? — —
2. Other serious illness? — —

	AGE	C.O.D./or PRESENT HEALTH
3. Father		
4. Mother		
5. Siblings		
6. Offspring		

C. PHYSICAL FINDINGS:

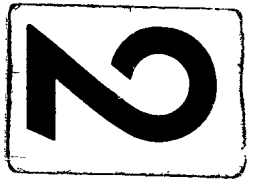
D. LABORATORY DATA:

Hb:
Hct:
WBC:
Urine:
NA:
K:
Cl:
CO_2
Steroids:
Gastric Analysis:
X-ray:

E. DIAGNOSIS:

F. SWEAT ELECTROLYTES:

Time:
Volume:
NA:
K:
Cl:



Room No.
Patient No.
Name
Address
City

Phone
Sex Age Race
Micro Macro

Out
Clinic
Private
Test No:
Date:
Time:

DIAGNOSES:

1.
2.
3.

	Time	Vol.	NA	K	Cl		
Saliva							
Sweat							

FILE CARD

APPENDIX 2

Date Analysis / Analysis Sheet SPECIMEN IDENT.	Test No.	Specimen Label	Vol. of Spec.	MICROTITRATION CHLORIDE		Dilution	FLAME PHOTOM SODIUM						
				TITER	MEQ/L		Low Known Conc.	Low Known Reading	High Known Conc.	High Known Reading	Unknown Reading	Unknown Conc.	Low Known

ANALYSIS FORM

APPENDIX 3

2

e Analysis / alysis Sheet / ECIMEN IDENT.	Test No.	Specimen Label	Vol. of Spec.	MICROTITRATION CHLORIDE		Dilution	FLAME PHOTOMETER													
							SODIUM						POTASSIUM							
				TITER	MEQ/L		Low Known Conc.	Low Known Reading	High Known Conc.	High Known Reading	Unknown Reading	Unknown Conc.	Low Known Conc.	Low Known Reading	High Known Conc.	High Known Reading	Unknown Reading	Unknown Conc.		

ANALYSIS FORM

APPENDIX 3

APPENDIX 4

EXOCRINOLOGY LABORATORY CODE SHEET FOR DATA PROCESSING

1-7 Hospital Number
(Y= not recorded or none)

8-11 Test Number

12-13 Month of test

14-15 Day of test
(Y= not recorded)

16 Previous Tests:

1. 1st Data
2. Previous Data (Pilocarpine)
3. Previous Data (Bag)
4. Part of serial study
Y. Not recorded

17 If Serial Study, Which Number

1. Use appropriate Number
X. No serial study
Y. Not recorded

18 Genetic Data

1. Other members of family studied
2. All members of family studied
3. Known cystic fibrosis family
X. No family data
Y. Not recorded

19-20 Age of Patient

X. Less than one year
Y. Not recorded

21 Sex and Race

1. Male - white
2. Male - nonwhite
3. Female - white
4. Female - nonwhite
5. Sex not recorded
6. Race not recorded
7. Neither recorded

22. Source of Patient

1. University Hospital-inpatient-clinical
2. University Hospital-inpatient-private
3. University Hospital-outpatient
4. Ohio Tuberculosis Hospital-inpatient
5. Outpatient
6. Normals
7. North American Aviation
Y. Not recorded

23-25 Sample size (In ml. to nearest 0.01 mg.)

X. Dry Sponge
Y. Not recorded

26-27 Test Collection Time (Time in Minutes)

Y. Not recorded

28. Source of Sweat

1. Forearm
2. Leg
3. Trunk
4. Facial
5. Axillary
Y. Not recorded

29 Collection Technique

1. Pilocarpine Iontophoresis - whole sweat
2. Pilocarpine Iontophoresis - elution
3. Bag Method
4. Direct
Y. Not recorded

30-33 Sweat Sodium in meq/l.

1 - 0 --Record as listed to nearest 0.1 mEq/l
X-- Lab error or not analyzed(33)
Y --Dry Sponge or not enough for analysis

34-36 Sweat Potassium in mEq/l.

1-0 --Record as listed to nearest 0.1 mEq/l
X --Lab error or not analyzed
Y --Dry sponge or not enough for analysis

37-40 Sweat Chloride in mEq/l.

1 - 0--Record as listed to nearest 0.1 mEq/l
X--Lab error or not analyzed (40)
Y--Dry sponge or not enough for analysis

41. Analytical Method

1. Chloride Macro
2. Chloride Micro- only
3. Chloride Micro Flame simultaneously
4. Chloride Micro Flame later
Y. Not recorded

42-44 Serum Sodium in mEq/l.

1 -0--Record as listd to nearest 1.0 mEq/l
X--Not measured within 48 hours of test
Y--Not recorded

45-46 Serum Potassium in mEq/L

1 -0--Record as listed to nearest 1.0 mEq/l
X--Not measured within 48 hours of test
Y--Not recorded

47-49 Serum Chloride in mEq/l

1 -0--Record as listed to nearest 1.0 mEq/l
X--Not measured within 48 hours of test
Y--Not recorded

50-51 URINE STEROIDS

17-Keto Steroids

1 -0--Record average of 3 values to nearest 1.0 milligram
X--Not measured within ± 1 month of test
Y--Not recorded

52-53 17-Hydroxy Steroids

1 -0--Record as average of 3 values to nearest 1.0 mg.
X--Not measured within ± 1 month of test
Y--Not recorded

54. Blood Type

1. O+
2. O-
3. AB+

4. AB-
5. A+
6. A-
7. B+
8. B-
Y. Not recorded

55. Diet

1. Unrestricted
2. 0-500 mg. NA
3. 0.50 - 2.5 gm. NA
4. Low calcium
5. Low fat
6. Low cholesterol
7. Ulcer type - all types
8. Gluten free
9. Low calorie
Y. Not recorded

56. Therapy

1. Mercurials
2. Thiazides
3. Both
4. Digitalis
5. NH_4 Cl
6. Steroids
7. ACTH
8. Antibiotics
9. Narcotics
0. Anticholinergics
X. None
Y. Not recorded

69. Amino Acid Composition

Y. Not run

70. Saliva

1. Normal electrolytes
2. Abnormal electrolytes
3. Protein electrophoresis
X. Collected
Y. Not collected

71. Aldosterome

72. Year of sweat test

1. 1959
2. 1960
3. 1961
4. 1962
5. 1963

I B M DATA SHEET

EXOCRINOLOGY LABORATORY IBM SHEETS

PATIENT'S NAME _____

HOSPITAL NUMBER 1 2 3 4 5 6 7

TEST NUMBER 8 9 10 11

TEST DATE
MONTH 12 13
DAY 14 15

PREV. TESTS 16

SERIAL STUDY 17

GENETIC DATA 18

AGE 19 20

SEX & RACE 21

PT. SOURCE 22

SAMPLE SIZE 23 24 25

TEST TIME 26 27

SOURCE 28

TECHNIQUE 29

SWEAT NA 30 31 32 33

SWEAT K 34 35 36

SWEAT CL 37 38 39 40

METHOD 41

SERUM NA 42 43 44

SERUM K 45 46

SERUM CL 47 48 49

17-KETO 50 51

17-HYDROXY 52 53

BLOOD GROUP 54

DIET 55

THERAPY 56

DIAGNOSIS NO. 1 57 58 59

DIAGNOSIS NO. 2 60 61 62

DIAGNOSIS NO. 3 63 64 65

DIAGNOSIS NO. 4 66 67 68

APPENDIX 5

I B M DATA SHEET

PATIENT'S NAME _____

HOSPITAL NUMBER 1 2 3 4 5 6 7

TEST NUMBER 8 9 10 11

TEST DATE: MONTH 12 13 DAY 14 15

PREV. TESTS 16

SERIAL STUDY 17

GENETIC DATA 18

AGE 19 20

SEX & RACE 21

IT. SOURCE 22

SAMPLE SIZE 23 24 25

TEST TIME 26 27

SOURCE 28

TECHNIQUE 29

SWEAT NA 30 31 32 33

SWEAT K 34 35 36

SWEAT CL 37 38 39 40

METHOD 41

SERUM NA 42 43 44

SERUM K 45 46

SERUM CL 47 48 49

17-KETO 50 51

17-HYDROXY 52 53

BLOOD GROUP 54

DIET 55

THERAPY 56

DIAGNOSIS NO. 1 57 58 59

DIAGNOSIS NO. 2 60 61 62

DIAGNOSIS NO. 3 63 64 65

DIAGNOSIS NO. 4 66 67 68

AMINO ACIDS 69

SALIVA 70

71

72

73

74

75

76

77

78

79

80

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